Effect of Brucellosis Vaccination and Dehorning on Transmission of Bovine Leukemia Virus in Heifers on a California Dairy

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ABSTRACT

Brucellosis vaccination and dehorning were examined for an association with bovine leukemia virus (BLV) infection in heifers on a California dairy between April 1984 and June 1987. Between December 1985 and June 1986, weaned heifers were dehorned using the gouge method at the time of brucellosis vaccination. Using logistic regression, the estimated probability for a nondehorned heifer to seroconvert within three months after brucellosis vaccination (0.08) was significantly less than that for heifers dehorned after a noninfected heifer (0.46) or than that for heifers dehorned after an infected heifer (0.85) ($p = 0.039$ and $p < 0.001$, respectively). To evaluate risk of transmission by brucellosis vaccination, which was usually done within one month postweaning, cumulative proportions of heifers remaining uninfected were computed among heifers that did not seroconvert three months after dehorning. Because results of a Cox model analysis indicated that groups of heifers were 6.6 times more at risk of becoming infected if placed in pens holding gouge-dehorned heifers (where prevalence varied between 50 and 70%) ($p < 0.001$) than other groups placed in pens without gouge-dehorned heifers (where prevalence varied between 10 and 30%), cumulative proportions of heifers remaining uninfected were computed for each type of group. The cumulative proportion of heifers remaining uninfected from weaning to first calving was 0.60 for the high prevalence group and 0.96 for the low prevalence group. No change in slope of cumulative proportions was observed before and after one month postweaning, suggesting that brucellosis vaccination was not an effective means of transmission. Results of this study indicate that bovine leukemia virus infection could be reduced from 80% to 4% in heifers between the time of weaning to calving by altering dehorning methods.

RÉSUMÉ

D'avril 1984 à juin 1987, la vaccination contre la brucellose et l'écornage à la gouge furent étudiés comme facteur possible de la transmission du virus de la leucose bovine chez des taures dans un troupeau laitier de Californie. Entre décembre 1985 et juin 1986, au moment de la vaccination contre la brucellose, les taures furent écornées à la gouge en utilisant la régression logistique, la probabilité estimée pour une taure non-écornée de séroconvertir en deça de trois mois, à la suite de la vaccination (0,08), était significativement moindre que pour une taure écornée après une taure non-infectée (0,46) ($p = 0,039$) ou une taure écornée après une taure infectée (0,85) ($p < 0,001$).

Pour évaluer le risque de transmission par la vaccination qui était habituellement fait dans le mois suivant le sevrage, les proportions cumulatives des taures qui demeurèrent non-infectés furent calculées parmi les taures qui ne seroconvertirent pas dans les trois mois suivant l'écornage.

À cause des résultats obtenus par la régression de Cox indiquant que les groupes de taures étaient 6,6 fois plus à risque de devenir infectées si elles étaient placées dans un parc avec des taures écornées à la gouge (ou la prévalence variait entre 50 et 70%) ($p < 0,001$) que les groupes placés dans les parcs contenant les taures non-écornées à la gouge (ou la prévalence variait entre 10 et 30%), les proportions cumulatives des taures demeurant non-infectées furent calculées pour chaque groupe. La proportion cumulative des taures demeurant non-infectées du sevrage au premier vêlage était de 0,60 pour le groupe à forte prévalence et de 0,96 pour le groupe à faible prévalence.

Aucun changement ne fut observé dans la pente des proportions cumulatives avant et après le mois suivant le sevrage, suggérant que la vaccination n'était pas un facteur important de transmission de la maladie. Les

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résultats de cette étude indiquent que le danger d'infection par le virus de la leucose bovine peut être réduit de 80% à 4% chez les taures entre le sevrage et le premier vêlage en changeant les méthodes d'écornage.

INTRODUCTION

Iatrogenic transmission of bovine leukemia virus (BLV) in dairy heifers has been proposed to occur through dehorning, vaccination and ear tattooing procedures (1-3). Use of the gouge dehorner was found to be connected to transmission of the virus when dehorned areas were not cauterized and when the instrument was not rinsed in disinfectant solution between animals (3). Ear tattooing was found also to transmit BLV (1,2). Rinsing of the tattooing die after each use, however, decreased the percentage of newly infected sheep only by two-thirds (2). Similarly, common-needle vaccination was not found to be associated with an increased risk of infection (4,5). Under the usual conditions experienced by dairy cattle, however, several factors may act simultaneously to alter the risk of infection. These may include the order in which cattle are dehorned and/or vaccinated and the prevalence of infection during and after dehorning and/or vaccination.

The objective of the present study was to quantify the effect of gouge-dehorning and brucellosis vaccination on the incidence rate of BLV infection in California dairy heifers from the age of weaning to first calving, after adjusting for the order in which cattle were dehorned and/or vaccinated and for the prevalence of infection during and after dehorning and/or vaccination.

MATERIALS AND METHODS

POPULATION EXAMINED

Heifers were studied on a dairy located in the Central San Joaquin Valley of California and managed as a typical California feedlot dairy (6). Calves were dehorned by electrocautery using a hot iron at about three months of age and were weaned at about six months of age. At about eight months of age, the few retained bulls were separated from the heifers and placed in a pen by themselves. Heifers were vaccinated subcutaneously with *Brucella abortus* (Strain 19) vaccine and tattooed for the purpose of brucellosis vaccination identification usually within one month postweaning. A few heifers were vaccinated, however, up to three months postweaning. The tattooing instrument was not disinfected between heifers and the same injection needle was used on all heifers vaccinated on a given day. In December 1985 and in April and June 1986, the dehorning procedure was changed and heifers were dehorned with the gouge method at the time of brucellosis vaccination. The dehorner and hemostats were not cleaned between heifers. Although all heifers were dehorned, either before or after weaning, heifers dehorned using the gouge method are referred to in the present study as the "dehorned" heifers. Heifers not dehorned using the gouge method are referred to as the "nondehorned" heifers.

COLLECTION OF DATA AND BLOOD SAMPLES

The following data were recorded between July 1984 and June 1987: date of birth, date and designation of pen changes, weaning and vaccination dates. Date of dehorning and/or vaccination and sequence in which heifers were dehorned and/or vaccinated were recorded between December 1985 and June 1987. Blood samples were collected every three months and sera were stored at -80° C.

DETERMINATION OF BLV INFECTION STATUS AND TIME OF INFECTION

To identify cattle infected with BLV, sera were examined for presence of gp-51 antibodies using the agar gel immunodiffusion test (Leukassay-B kit, Pitman-Moore Inc., Washington Crossing, New Jersey). The test procedure was slightly different from the protocol described by Miller and Van Der Maaten, and Nakajima *et al* (7,8). The agar-gel was made with 1% agarose and 8.5% NaCl in distilled water. Glass plates measuring 100 by 80 mm and containing 21 mL of agar were used. Wells were 4 mm in diameter and at a distance of 5 mm from each other. Antigen was placed in the central well, the reference positive serum in two opposite wells, and sera to be tested in the four remaining wells. Plates were incubated 48 h at 25° C in a humidified chamber before being viewed. A line of identity between antigen and antibody was interpreted as a positive result.

For heifers that became infected, the exact age at infection, expressed in days, could not be determined because heifers were not bled daily and because the serological test fails to detect infection until antibody concentration reaches a detectable level. Using the results from a previous study that estimated time-to-seroconversion following experimental BLV infection (9), it was assumed that infection, when viewed retrospectively, could have occurred between 110 days prior to the last negative test (t1) and seven days prior to the first positive test (t2). All possible ages at infection within that time interval (t1-110, t2-7) were assumed, *a priori*, to be equally likely. In other words, the prior probability function was assumed constant over that range of age values. Using the knowledge of time-to-seroconversion following experimental infection, the posterior probability function for the age at infection for an animal that seroconverted between t1 and t2 could be obtained, as described previously (9). Such functions allowed us to derive the "most likely" age at infection for a heifer that seroconverted and the probability of becoming infected before a specific time of interest (9).

DESCRIPTIVE STATISTICS

Monthly cumulative hazards of infection and monthly averages of daily prevalence rates were computed for the age groups 6 to 14 months and 15 to 23 months. To obtain monthly cumulative hazards, daily hazards were first obtained by summing conditional probabilities of becoming infected on a given day and dividing by the number of animals estimated to be at risk at the beginning of that day. Monthly cumulative hazards were derived by summing daily hazards over a given month.

To obtain monthly averages of daily prevalence rates, daily prevalence rates were computed by summing the

estimated number of infected animals in a day and dividing by the total number of animals present on that day. Monthly averages of daily prevalence rates were computed by summing daily prevalences over a given month and dividing by the number of days in that month.

Average prevalence and average monthly cumulative hazard were computed for all heifers that had not yet freshened.

FACTORS STUDIED AND STATISTICAL METHODOLOGY USED

To evaluate the association between gouge dehorning and BLV transmission, two approaches were used. First, the probability of seroconversion within three months after dehorning and/or brucellosis vaccination was examined. It was assumed that if heifers became infected at the time of dehorning and/or vaccination, gp-51 antibodies would be detected in their serum within the following three months (9). Several factors were examined for an association with that probability, namely: 1) whether a heifer was dehorned using the gouge method at the time of vaccination (coded as DEH = 1 for dehorned and DEH = 0 for not dehorned), 2) whether a heifer was dehorned immediately following an infected heifer, referred to as sequence of dehorning (coded as SEQ = 1 for dehorned after an infected heifer and SEQ = 0 for not dehorned after an infected heifer), and 3) the prevalence of infected heifers at the time of vaccination. Percentages of heifers that seroconverted within three months after brucellosis vaccination were determined for groups that were vaccinated only and for groups that were vaccinated and dehorned. Forward stepwise logistic regression was used to evaluate the effect of dehorning and sequence of dehorning on the probability of seroconversion within three months following vaccination, after adjusting for the prevalence of infected heifers at the time of vaccination. Computations were done using BMDPLR (10).

The second approach was to examine the age at infection for heifers dehorned and not dehorned. This design did not limit the observation span to three months after brucellosis vaccination and included all heifers present in the herd during the study period. The most likely age at infection was obtained for all heifers that seroconverted after weaning using the previously described method (9). Cumulative proportions of heifers remaining uninfected after weaning were compared for the dehorned and nondehorned groups using Breslow and Mantel-Cox statistics (11). Computations were done using BMDP1L (10).

Because all heifers were vaccinated after weaning, the association between brucellosis vaccination and BLV transmission could not be evaluated by comparing the probability of seroconversion within three months following vaccination in vaccinated and nonvaccinated heifers. The first approach used to examine the association between gouge dehorning and BLV transmission, therefore, could not be used for brucellosis vaccination. The second approach described above, however, could be used to make time-related comparisons. If brucellosis vaccination was an effective means of transmission, risk of infection would be expected to be higher within one to three months postweaning.

The Cox model was used to evaluate risk of infection from weaning to first calving (11). Because dehorning appeared to be very effective in transmitting BLV, dehorned heifers that seroconverted within three months following dehorning could have been infected because of dehorning and not through vaccination. Consequently, heifers seroconverting three months after dehorning were not included in the analysis. In addition, because dehorning appeared to be responsible for a significant increase in the incidence of BLV infection, it was not unexpected that prevalence of infection in pens holding dehorned heifers (50-70%) was greater than that in pens holding nondehorned heifers (10-30%). Therefore, it was hypothesized that noninfected heifers placed in a pen with dehorned heifers (COH = 1) would be at a greater risk of becoming infected than those placed in a pen with nondehorned heifers (COH = 0). Consequently, the factor "presence or absence of dehorned heifers in a pen" was considered as a variable to adjust for an indirect affect of dehorning when modelling the risk of infection through vaccination. Computations were done using BMDP1L (10).

In all survival analyses, the regression coefficient divided by its standard error was computed for each factor in the model. If the resulting ratio, considered to be a z-value, was equal or greater in absolute value than 1.96, it was interpreted as indicating a significant effect at or below the 5% level. A negative regression coefficient indicated that the factor level coded 0 favored infection, while the factor level coded 1 did not. Interpretation of a positive coefficient was the reverse.

RESULTS

Descriptive statistics of BLV infection in heifers 6 to 14 months old showed monthly cumulative hazards that increased sharply between September 1985 and June 1986, then decreased until October 1986 and then slowly increased until the end of the study (Fig. 1). Increased cumulative hazards accounted for an increased prevalence in that age group (Fig. 2) during the same period. As the 6 to 14 month old heifers aged, prevalence of infection increased in the 15 to 23 month old cohort, as depicted in Fig. 2. Initially, in the 15 to 23 month old cohort, monthly cumulative hazards were less than 2% of the heifers but increased up to 6% by the end of the study (Fig. 1). The overall average prevalence was 28.9% and the overall average monthly cumulative hazard was 2.5%.

Date of dehorning and/or vaccination and sequence in which heifers were vaccinated and/or dehorned were recorded between December 1985, and June 1987, for 77 noninfected heifers. The average percent of heifers seroconverting within three months was 5.1 (2/39) for those vaccinated only, and 59.5 (22/37) for those dehorned and vaccinated. One noninfected heifer was removed from the herd shortly after vaccination. A logistic regression analysis showed a significantly greater probability of seroconversion within three months after brucellosis vaccination for heifers dehorned than for heifers not

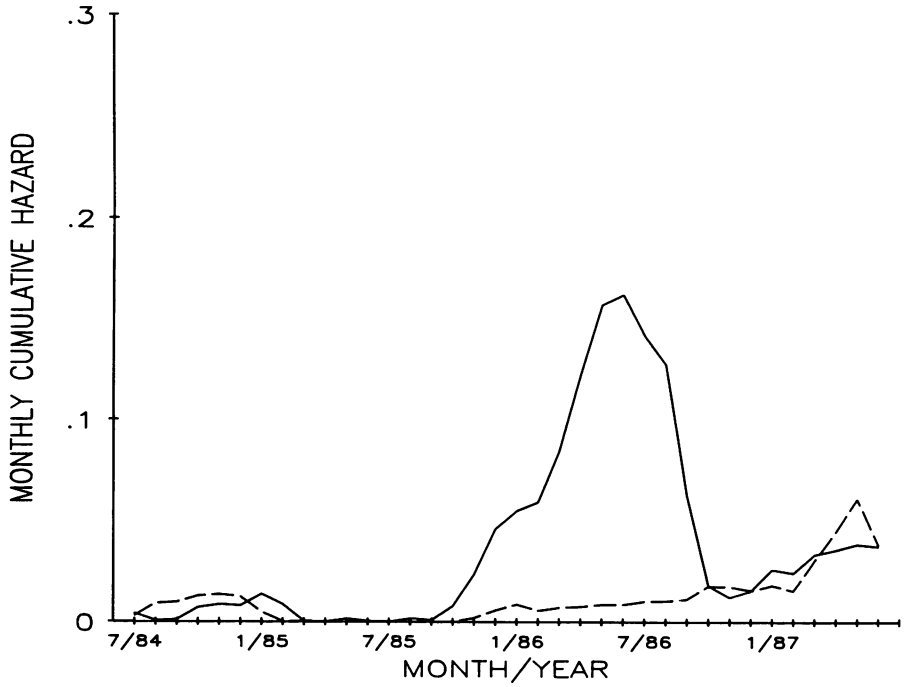

Fig. 1. Monthly cumulative hazards of BLV infection for cattle aged 6 to 14 months (_____) and 15 to 23 months (— — —) between July 1984 and June 1987.

dehorned ($p = 0.001$). Heifers dehorned after an infected heifer also had a greater probability of seroconversion than those dehorned after a noninfected heifer ($p = 0.039$). Prevalence of infected heifers at the time of vaccination, however, was not found to be associated with a change in the probability of seroconversion ($p = 0.728$). The resulting model for the probability of seroconversion was the following:

$$\frac{\exp(-2.485 + 2.318*DEH + 1.872*SEQ)}{1+\exp(-2.485 + 2.318*DEH + 1.872*SEQ)}$$

Using this model, estimated probabilities of seroconversion within three months after vaccination were 0.08, 0.46 and 0.85 among heifers vaccinated only, dehorned after a noninfected heifer, and dehorned after an infected heifer, respectively. Using the estimated probabilities as proportions of calves expected to become infected, the risks of infection attributable to dehorning after an infected heifer and after a noninfected heifer were 0.77 (0.85-0.08) and 0.38 (0.46-0.08), respectively.

When all the 163 noninfected heifers weaned during the study period were used to model age at infection, the cumulative proportion remaining uninfected was significantly greater for nondehorned than for dehorned ($p < 0.001$) (Fig. 3). Breslow and Mantel-Cox statistics were 70.76 and 80.29, respectively. A marked decline of the survival curve for dehorned heifers was observed within 80 days postweaning (Fig. 3), which included the time at which most heifers were dehorned.

For heifers that did not seroconvert within three months after dehorning, the Cox model was used to examine risk of infection over time. Analyses showed that heifers were 6.6 [exp(1.885*1)/exp(1.885*0)] times more at risk of infection if they were in a pen holding dehorned heifers than if they were not in a pen holding dehorned heifers ($p < 0$ .001). Cumulative proportions of heifers remaining uninfected were obtained for each group (COH = 0, COH = 1) using BMDP1L (10). Breslow and Mantel-Cox statistics for differences in the cumulative proportions of heifers remaining uninfected were 13.94 and 16.58, respectively ($p = 0.0002$ and $p < 0.0001$), indicating that fewer heifers not exposed to dehorned heifers became infected compared to those that were exposed. For noninfected heifers that were neither dehorned nor in contact with dehorned heifers, cumulative proportions remained above 96% (Fig. 4).

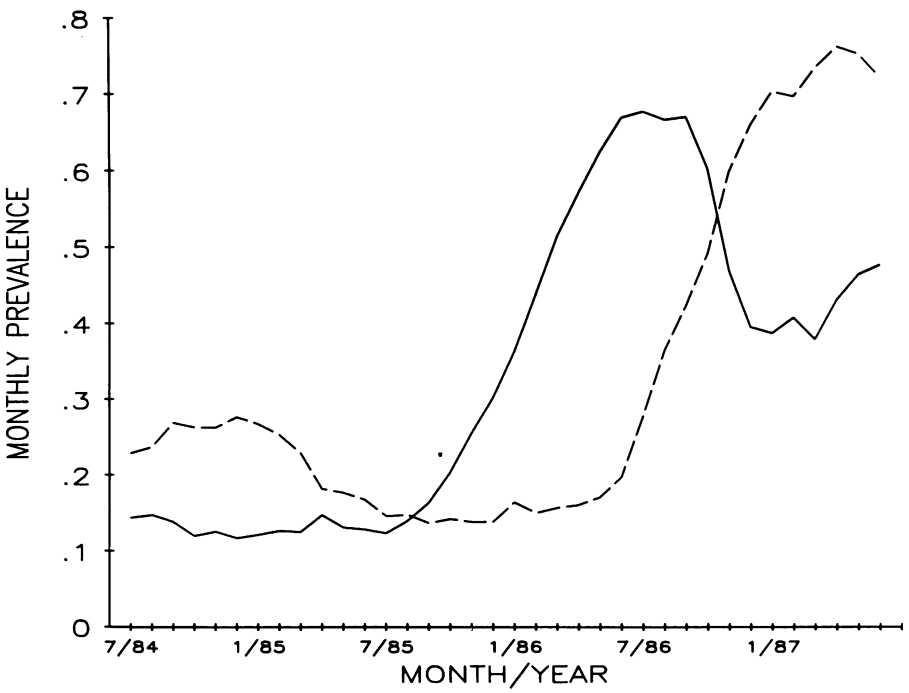

Fig. 2. Average monthly prevalence of BLV infection for cattle aged 6 to 14 months (_____) and 15 to 23 months (— — —) between July 1984 and June 1987.

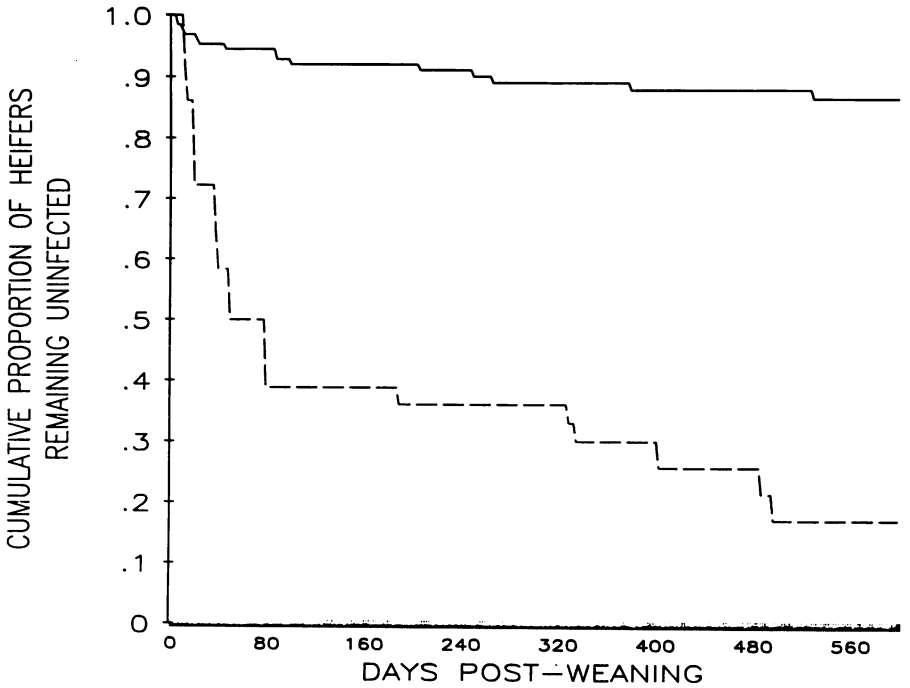

Fig. 3. Cumulative proportions of heifers remaining uninfected with BLV that were weaned between July 1984 and June 1987 and gouge-dehorned (– – – –) or not gouge-dehorned (_____).

Cumulative proportions decreased significantly to 60% for the heifers exposed to dehorned heifers (Fig. 4). No marked decline of the survival curves, however, was observed within one to three months following weaning, indicating that brucellosis vaccination was not an effective means of transmission.

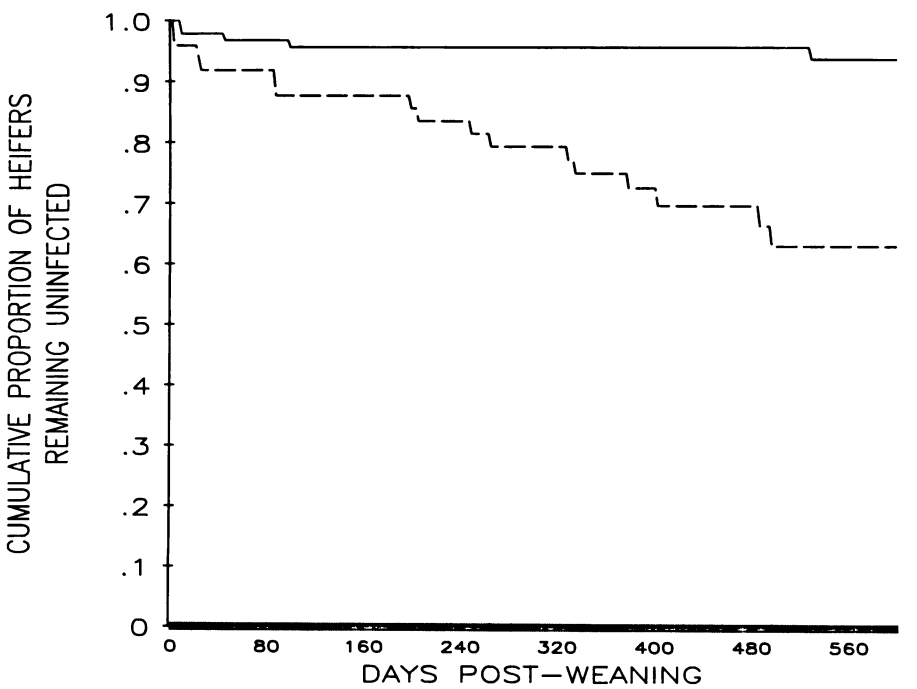

Fig. 4. Cumulative proportions of heifers remaining uninfected with BLV that were weaned between July 1984 and June 1987 and not placed in a pen with gouge-dehorned heifers (_____) or placed in a pen with gouge-dehorned heifers (– – – –).

DISCUSSION

Dehorning with a gouge dehorner was found to be strongly associated with transmission of BLV, increasing the risk of infection for vaccinated heifers from 8% up to 77%. The risk attributable to dehorning could vary between 77%, if all heifers were dehorned after infected ones, and 38%, if all heifers were not dehorned after infected ones. This result agrees with a previous report which found an increased risk of infection of about 86% following gouge-dehorning (3). Such a dehorning method, therefore, should not be used in dairies interested in controlling or eradicating BLV infection. It was suggested previously that transmission of BLV by dehorning could be through transfer of blood or tissue left on the dehorning instrument, because rinsing the dehorner in disinfectant between calves and cauterizing dehorned areas significantly reduced the probability of seroconversion (3). Such a hypothesis that transmission would occur during dehorning would be in agreement with the finding of the present study that a heifer had a greater probability of seroconverting if dehorning took place after an infected heifer than after a noninfected one.

Procedures employed during vaccination of cattle, which include injection of the vaccine, ear-tagging and tattooing, did not seem to be associated with the spread of BLV. Similar observations were made in another observational study (4). For heifers that did not seroconvert within three months after dehorning, the cumulative proportion remaining uninfected did not show any change of slope within one to three months postweaning (Fig. 4), as would be expected if brucellosis vaccination and tattooing contributed to BLV transmission. If gouge-dehorning was never used on this dairy, 96% of weaned and vaccinated heifers would be expected to enter the milking herd uninfected (Fig. 4) and, therefore, the overall herd prevalence would be expected to decrease, as observed in another study (12).

In two experimental studies, transmission of BLV by ear tattooing was found to be an effective way to infect sheep (1,2). In one of those studies, 2 mL of blood from an infected cow was spread on the tattooing instrument (2) and in the other, the tattooing instrument was used repeatedly on the same infected calf (1) before its use on noninfected sheep. The fact that results of experimental studies do not agree with observations of natural transmission may be due to excessive blood contamination of tattooing dyes used in experimental studies. Under field conditions, tattooing may not consistently rupture blood vessels to a point where infected lymphocytes are present in sufficient number on the tattoo prongs. In the present study, the two nondehorned heifers that seroconverted within three months after vaccination were not tattooed after an infected heifer, possibly indicating that the heifers did not become infected during vaccination procedures. Because of the apparent low risk of infection during brucellosis vaccination, disinfecting the tattoo instrument after each use would not seem to be economically justified.

An indirect effect of dehorning on risk of BLV transmission was observed. Cumulative proportions of heifers remaining uninfected were lower for those placed in pens with dehorned heifers than for those placed in pens with nondehorned heifers (Fig. 4). The slope of the survival curve for the group not placed with dehorned heifers was very similar to the one observed in another study of heifers 7 to 16 months old (4). In that study, cumulative proportions of heifers remaining uninfected decreased sharply and significantly to 40% when bred heifers were placed with older cattle that were 90 to 100% infected (4). The relative risk of infection for bred heifers was 6.7 times greater than in the 7 to 16 month old heifers (4). In the present study, cumulative proportions of heifers remaining uninfected stayed above 60% for heifers placed in a pen with dehorned heifers (Fig. 4) and the relative risk of infection was 6.6 times greater than for heifers not placed in a pen with dehorned heifers. These results suggest that the risk of infection is related to the prevalence of infection in cattle sharing the pen and provide some evidence for direct transmission by contact. If cattle were placed in a pen with prevalence ranging from 50 to 70%, about 60% would be uninfected when starting their first lactation compared to 96% if they were placed in a pen with prevalence ranging from 10 to 30% (Fig. 4).

The relationship between risk of infection and prevalence of infection in a pen did not seem to be linear. For an increase in pen prevalence from 0 to 30%, about 4% (100%-96%) of heifers could be infected at their first lactation and for the same amount of increase from 30 to 60%, about 36% more [(100%-60%) — (100%-96%)] could be infected. Although these numbers are approximate, a nonlinear relationship between risk of infection and prevalence would be in agreement with the theory of infectious diseases, which describes the spread of an infectious agent by contact (12). Knowledge of a prevalence threshold below which transmission is unlikely to occur would be of benefit in defining control alternatives.

In conclusion, the results of this study suggest that a substantial amount of BLV infection can be reduced (from 80% to 4% in this dairy) by not dehorning heifers using a gouge dehorner. No evidence, however, was found to suggest modifications of common brucellosis vaccination procedures. Further research on the modeling of BLV transmission should be fruitful and helpful in the planning of control programs.

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